LSC in Practice

Rat Blood — Severe Color Problems

Problem

A researcher contacted PerkinElmer's Application Laboratories with a request for help in overcoming a severe color problem she was experiencing when attempting to solubilize rat blood (containing 2 mL heparin per 20 mL blood). The researcher had been using 1.0 mL of PerkinElmer's Soluene®-350 with 0.4 mL rat blood when the color problem was noticed and she realized that ^{3}H determination would be difficult and error prone.

Discussion

For this investigation, we tested solubilization with the following PerkinElmer solubilizers:

1. Soluene-350 (part number 6003038)
2. SOLVABLE™ (part number 6NE9100)
3. Hyamine Hydroxide® (part number 6003005)

Basically, the use of either Soluene and Hyamine Hydroxide® is not recommended. Even with attempted color removal using hydrogen peroxide, there was still too much color present to obtain proper counting results. However, the use of SOLVABLE resulted in a final mixture that was low in color.

We tried a few different methods but the recommended procedure is as follows:

1. To 0.5 mL rat blood add 1.0 mL SOLVABLE.
2. Incubate the samples for one hour at 50 °C to 55 °C. Sample appearance will change from red to brown/green.
3. Add 0.1 mL of 0.1 M EDTA-di-sodium solution. This will help reduce foaming which occurs when the peroxide is added.
4. Add 0.3 mL to 0.5 mL (we used 0.5 mL) 30% hydrogen peroxide in 0.1 mL increments. Shake and agitate gently between additions to allow reaction and foaming to subside.
5. Allow to stand for 10 to 15 minutes to allow reaction to complete and then cap tightly.
6. Incubate in the oven at 50 °C to 55 °C for one hour, venting any slight pressure occasionally. The color will change from brown/green to light yellow.
7. Cool to room temperature and add 10 mL to 15 mL of scintillation cocktail.
8. Light and temperature adapt for one hour in the counter prior to counting.

Note: During step 6, there will be some final frothing and it is essential that the vials are tightly capped to prevent overflow. The occasional venting means about every 20 minutes.

Using this method, we prepared several samples and then added the following cocktails:

CPM in 0 to 18.6 keV window

Table 1:

Time	Pico-Fluor™	40 Hionic-Fluor™
2 minutes	97 CPM	341 CPM
5 minutes	52 CPM	113 CPM
10 minutes	26 CPM	80 CPM
20 minutes	22 CPM	41 CPM
1 hour	19 CPM	23 CPM
tSIE (10 mL)	198 (= 26%)	189 (= +25%)
tSIE (15 mL)	238 (= 29%)	218 (= +27%)
Appearance	Clear, slightly	Slightly hazy and yellow slightly yellow*

* *If 15 mL of PerkinElmer's Hionic-Fluor (part number 6013319) are added, a clear mixture is obtained. Counted on a PerkinElmer Tri-Carb® 2550TR/AB operated at 15 °C.*

Recommendation:

We also tried the method with 0.5 mL rat blood and 0.5 mL SOLVABLE but the end result was more colored and therefore we recommend **2 parts SOLVABLE to 1 part rat blood**.

The procedure listed above will minimize the colorization problems and give the researcher a reliable and reproducible system for counting her samples.

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